

Length of concentrate finishing affects the fatty acid composition of grass-fed and genetically lean beef: an emphasis on *trans*-18:1 and conjugated linoleic acid profiles

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Intensively finishing cattle on a high-grain diet is generally used to enhance marbling, whereas extensively finishing on grass is known to provide improved muscle fatty acid profiles. The objective of this study was to evaluate to what extent intensive concentrate finishing (0, 1 or 2 months) can be combined with forage feeding without negatively affecting the fatty acid profile of genetically lean animals. Bulls from the 'Asturiana de los Valles' breed were reared under grazing conditions with/without final finishing on a barley-based concentrate: 0 months (control; n = 7), 1 month (n = 10) and 2 months (n = 7). Yearling bulls were slaughtered commercially at an average live weight of 516 ± 9.8 kg. Increasing the finishing time on concentrate significantly increased the saturated and monounsaturated fatty acids, whereas polyunsaturated fatty acids (PUFAs) tended to decrease and it was not possible to increase the long-chain PUFA content in muscle tissue of this breed. An increase was observed for total trans-18:1 (average 5.5% with grain v. 3.7% for grass). The 11t-18:1/10t-18:1 ratio was significantly higher in grass-fed (average 8.1) compared with grain-finished animals (average 1.1). Grass or limited concentrate finishing reduced the n-6/n-3 ratio in muscle tissue (average 3.6 for 0 and 1 month, and 4.9 for 2 months on grain finishing). The beef was within or close to the recommended values for human consumption (i.e. polyunsaturated/saturated > 0.45, n-6/n-3 < 4.0), and total trans-FA content was low. However, finishing increased the content of undesirable trans-18:1 and conjugated linoleic acid isomers, particularly after 2 months, whereas grass finishing was judged to provide a healthier beef fatty acid profile.

Keywords: beef, CLA, concentrate, grass, vaccenic acid

Implications

Diet is one of the most important factors influencing fat content and fatty acid composition in beef. Pasture-fed beef offers lean meat with a more desirable fatty acid composition for human consumption (n-3, conjugated linoleic acids (CLAs)). However, extensive feeding can be quite compromising in double-muscling animals due to their inherent low fat content. In order to deposit some carcass and intramuscular fat, these animals are typically finished on concentrate for few months before slaughter. However, results indicate that finishing has a major impact on the quality and the content of *trans*-18:1 and CLA isomer composition,

which was negatively affected mainly with a longer period (2 months) of concentrate finishing.

Introduction

Beef fat content and composition depend primarily on breed and feeding (Rule *et al.*, 1995; Aldai *et al.*, 2006). For instance, in the 'Asturiana de los Valles' breed from northern Spain, the frequency of animals exhibiting doubled muscling has increased over the last decade to the point now where most of the animals of this breed are genetically homozygous (*mhl/mhl*) or heterozygous (*mhl/+*) for the gene responsible for muscular hypertrophy (Grobet *et al.*, 1998; Pérez, 2005). Consequently, the beef obtained is very lean and the fatty acids profile can be affected (Aldai *et al.*, 2007a and 2007b). A high fat content in muscle is associated with

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increased neutral lipids (triacylglycerides), whereas phospholipid levels remained relatively constant (Rule *et al.*, 1995).

Grain-based diets contain relatively high levels of linoleic acid, which can lead to unfavourable n-6/n-3 ratios (Aldai *et al.*, 2008b) and *trans*-18:1 profiles (Aldai *et al.*, 2008b; Kraft *et al.*, 2008) in beef. The 10*trans* (*t*)-18:1 accumulates in intensively or concentrate-finished beef (Purchas *et al.*, 2005; Leheska *et al.*, 2008) and this has been associated with increased atherogenicity in animal models (Bauchart *et al.*, 2007; Roy *et al.*, 2007). Forages are good sources of linolenic acid that leads to an enhanced n-3 polyunsaturated fatty acid (PUFA) content in beef products with a more desirable fatty acid composition for human health (Gleissman *et al.*, 2010). Moreover, rumenic acid (RA, 9*cis* (*c*), 11*t*-18:2) and its precursor vaccenic acid (VA, 11*t*-18:1) have been found to accumulate in forage-finished ruminants (Dugan *et al.*, 2007; Kraft *et al.*, 2008; Alfaia *et al.*, 2009) and their consumption has been linked to a number of potential health benefits (Bauchart *et al.*, 2007; Field *et al.*, 2009; Bassett *et al.*, 2010).

Designing diets and production systems for lean breeds (i.e. double-muscle animals) poses a challenge in terms of their fat content and composition. Extensive production systems (i.e. forage feeding) can be positive for beef fatty acid composition, but a low fat level can lead to a negative classification of carcasses based on the European carcass classification system (Commission Regulation, 1981; Council Regulation, 1981). Therefore, it is necessary for cattle with high lean growth potential (see review by Arthur, 1995) to be fattened on high-energy concentrate diets in order to deposit sufficient carcass and marbling fat (Nuernberg *et al.*, 2005; Martínez *et al.*, 2010). Thus, the objective of the present study was to evaluate the length of intensive concentrate finishing (0, 1 or 2 months) that should be combined with forage feeding to produce a carcass with sufficient fat to pass the European classification rating and still retain a fatty acid profile with a positive health image.

Material and methods

Animals and management

Twenty-four spring-born suckling male calves from 'Asturiana de los Valles' Spanish beef breed, heterozygous for the gene responsible for muscular hypertrophy (*mhl* +; Grobet *et al.*, 1998), were reared with their mothers under grazing conditions until weaning in November. The average live weight of calves at weaning was 254 ± 9.5 kg and they were reared extensively until they reached 472 ± 13 kg. Pastures were mainly composed of ryegrass (*Lolium perenne*) and clover (*Trifolium repens*), and during winter when grass availability was low, animals were confined and fed hay. From mid-May, some bulls continued under extensive management (pasture grazing without concentrate; control, $n = 7$), whereas the remaining bulls were finished on a barley-based concentrate for either one (1 mo; $n = 10$) or 2 months (2 mo; $n = 7$) in the housing facilities of the SERIDA Research Institute (Asturias, northern Spain). The transition

from grass to concentrate was performed over a 4 to 5-week period after which concentrate (84% barley meal, 10% soya meal, 3% vegetable oil and 3% supplement composed of a mineral/vitamin/oligoelement premix) and barley straw were fed *ad libitum*. The 4 to 5-week transition period from grass to concentrate was introduced to extend the production system *per se* as the main objective was to maximize the utilization of natural resources. During this transition period, animals were provided with 1.5 to 2 kg of concentrate per head per day. The proximate chemical and fatty acid composition of the concentrate was previously reported by Aldai *et al.* (2007b), and grass composition was indicative of its good quality (22.9% protein, 27.2% ADF, 38.3% NDF and 2.47 Mcal/kg of metabolizable energy on dry matter basis).

Yearling bulls were slaughtered commercially at an average live weight of 516 ± 9.8 kg.

Sample collection

Grass and concentrate were sampled every 2 months and 3 weeks, respectively. Samples were freeze-dried and ground through a 1 mm screen. Fatty acid methyl esters (FAMES) were prepared according to Sukhija and Palmquist (1988) and analysed according to Dugan *et al.* (2007).

After slaughter, carcasses were chilled for 24 h at 3°C and left rib joints from the sixth to the ninth ribs were removed and transported to the laboratory. A *longissimus thoracis* steak was dissected from the eighth rib, vacuum packed and frozen at -80°C for subsequent fatty acid analyses.

Fatty acid composition of muscle

Lipids were extracted from 1 g of freeze-dried muscle using a mixture of chloroform-methanol (1:1, v/v) (Kramer *et al.*, 1998). Lipid aliquots (10 mg) from each muscle sample were methylated separately using acidic (methanolic HCl) and basic (sodium methoxide) reagents (Kramer *et al.*, 2008). Internal standard (1 ml of 1 mg 23:0 methyl ester per mL toluene) was added before the addition of the methylating reagent. The FAMES were analysed using GLC and Ag^{+} -HPLC equipment and methods outlined by Cruz-Hernandez *et al.* (2004). *Trans*-18:1 isomers were analysed using two complementary GLC temperature programmes (Kramer *et al.*, 2008). In representative samples, silver-ion solid phase extraction (Ag^{+} -SPE) cartridges were used to separate fractions of saturated FAMES, mono-*trans* FAMES plus the *t/t* isomers of conjugated linoleic acid (CLA), mono-*cis* FAMES plus the *d/t* isomers of CLA, dienoic FAMES, trienoic FAMES and tetraenoic FAMES to confirm peak identification (Kramer *et al.*, 2008). Reference standards 463, 603 and U-59 from Nu-Chek Prep Inc. (Elysian, MN, USA) were used. Phytanic acid was obtained from Matreya Inc. (Pleasant Gap, PA, USA). The UC-59 standard contained all possible geometric and positional isomers of 8,10-CLA, 9,11-CLA, 10,12-CLA and 11,13-CLA. Branched-chain fatty acids (BCFA) were identified using GLC reference standard BC-Mix1, purchased from Applied Science (State College, PA, USA). The *trans*-18:1, CLA and other diene isomers not included in standard mixtures were identified by their retention times and elution

orders as reported elsewhere (Cruz-Hernandez *et al.*, 2004 and 2006; Kramer *et al.*, 2008; Rego *et al.*, 2009). The FAMES were quantified using chromatographic peak area and internal standard (23:0 ME)-based calculations. The contents of FAME were expressed on an mg per 100 g of fresh meat and on the % of total FAME basis.

Statistical analysis

Individual, groups and ratios of FAME from *longissimus thoracis* muscle were analysed as a one-way ANOVA using the PROC MIXED procedure of SAS (SAS Institute, 2001) with concentrate finishing (0, 1 or 2 months) as the main effect. The LSMEANS and PDIF options were used to generate least square means and to compare treatments (*F*-test protected least significant difference). Significance was declared at $P < 0.05$.

Results

The lipid content (measured as total FAME) of the two feedstuffs utilized in the present study were 21.9 and 53.9 mg/g of dry matter for grass and concentrate, respectively (Table 1). In both, major fatty acids representing >90% of the total FAME included palmitic (16:0), stearic (18:0), oleic (9c-18:1), linoleic (18:2n-6) and linolenic (18:3n-3) acids. The percentage of 16:0 was similar in grass and concentrate. Grass samples were lower in 18:0, 9c-18:1 and 18:2n-6, but clearly higher in 18:3n-3.

Table 1 Total FAME and fatty acid composition (mean and standard error of the mean) of grass and concentrate samples

Feed composition	Grass ($n = 10$)		Concentrate ¹ ($n = 4$)	
FAME (mg/g DM)	21.90	2.012	53.88	0.989
Fatty acid (FAME, %)				
12:0	0.070	0.004	0.012	0.003
14:0	0.252	0.010	0.176	0.003
15:0	0.107	0.010	0.047	0.002
16:0	12.81	0.923	15.32	0.081
7c-16:1	2.539	0.047	0.044	0.005
9c-16:1	0.153	0.004	0.106	0.003
17:0	0.150	0.016	0.097	0.001
9c-17:1	0.042	0.004	0.050	0.001
18:0	1.443	0.206	3.191	0.011
9c-18:1	1.875	0.167	19.82	0.032
11c-18:1	0.317	0.020	1.140	0.004
18:2n-6	13.80	0.367	52.58	0.174
20:0	0.324	0.053	0.334	0.002
9c-20:1	0.066	0.018	0.031	0.001
11c-20:1	0.047	0.002	0.385	0.003
18:3n-3	61.61	2.322	5.896	0.083
20:2n-6	0.048	0.004	0.061	0.001
22:0	0.487	0.098	0.358	0.004
13c-22:1	0.021	0.003	0.044	0.003
24:0	0.570	0.136	0.212	0.009
15c-24:1	0.019	0.003	0.030	0.002

FAME = fatty acid methyl ester; DM = dry matter.

FAME > 0.02% was reported.

¹The barley-based concentrate consisted of 84% barley meal, 10% soya meal, 3% fat and 3% supplement composed of a mineral/vitamin/oligoelement premix.

Longissimus muscle from bulls fed grass only had lower total FAME contents than from bulls finished on concentrate for 2 months (0.5% and 1.0%, respectively; $P < 0.05$), whereas bulls finished on concentrate for 1 month had intermediate FAME levels (0.8%; Table 2).

Saturated and BCFA, and plasmalogenic lipids

Muscle from bulls finished on concentrate for 2 months had the highest total saturated fatty acid (SFA) content (448 mg/100 g of meat; $P < 0.05$; see summary Table 2). Bulls finished for 1 month on concentrate had an intermediate level (316 mg/100 g of meat), whereas bulls finished on grass had the lowest content (197 mg/100 g of meat). On a percentage basis, the trend was similar ($P < 0.05$) with 41% SFA after 2 months, 38% after 1 month and 36% when pasture fed (Table 3). With regard to individual SFAs, percentages of 12:0, 14:0 and 16:0 were higher ($P < 0.05$) in meat from concentrate-finished bulls, whereas longer chain SFAs (21:0, 22:0) were higher ($P < 0.05$) in grass-finished bulls. Finishing strategy did not influence the percentage of BCFA (individual or total; $P > 0.05$).

Finishing affected the percentages of plasmalogenic lipids, identified as their dimethylacetals (DMA) after methylation, and their fragmentation products produced during GLC analysis (alk-1-enyl methyl ethers, AME). Plasmalogenic

Table 2 Total FAME content (mg/100 g meat) and summary of fatty acids of nutritional interest (mg/100 g meat) and ratios of essential fatty acids from *longissimus thoracis* of bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing

Fatty acids (mg/100 g)	Control	1 mo	2 mo	s.e.m.	P-value
Σ FAME	547.0 ^b	812.6 ^{ab}	1055 ^a	113.7	0.027
Σ SFA	197.2 ^b	316.4 ^{ab}	447.6 ^a	56.32	0.028
Σ cis-MUFA	126.4	211.8	309.8	25.330	0.059
Σ trans-MUFA	24.51	55.07	62.80	6.06	0.053
10t-18:1	1.637 ^b	23.86 ^a	22.80 ^a	5.324	0.015
11t-18:1	14.20	16.26	20.54	5.344	0.713
11t-18:1/10t-18:1	8.134 ^a	0.780 ^b	1.299 ^b	0.491	<0.001
Σ MUFA	156.4	273.4	355.9	52.73	0.057
Σ trans-FA	26.75	60.95	66.10	10.21	0.053
Σ PUFA	130.5	155.3	158.1	9.153	0.104
Σ n-6 PUFA	99.24 ^b	123.9 ^a	130.6 ^a	7.781	0.031
18:2n-6	76.52 ^b	95.25 ^{ab}	103.3 ^a	6.538	0.033
Σ n-3 PUFA	31.23	31.45	27.45	2.290	0.396
18:3n-3	18.20 ^a	16.32 ^a	12.49 ^b	1.348	0.026
Σ n-6 HUFA	22.99	28.65	26.88	1.200	0.162
Σ n-3 HUFA	13.04	15.12	15.00	0.742	0.463
n-6/n-3 PUFA	3.292 ^b	4.010 ^{ab}	4.872 ^a	0.331	0.014
n-6/n-3 HUFA	1.862	1.906	1.822	0.069	0.879
P/S	0.704	0.604	0.414	0.048	0.081
Σ CLA	2.838	3.637	5.326	0.984	0.233
9c,11t-18:2	1.857	2.167	3.492	0.766	0.311

FAME = fatty acid methyl ester; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; HUFA = highly unsaturated fatty acid; P/S = polysaturated/saturated; CLA = conjugated linoleic acid.

Within a row, means without a common superscript differ ($P < 0.05$).

See Tables 3, 4, 5, 6 and 7 for grouping explanations.

Table 3 SFA and DMA composition (percentages) of longissimus thoracis from bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing

Fatty acids (%)	Control	1 mo	2 mo	s.e.m.	P-value
12:0	0.014 ^b	0.022 ^a	0.024 ^a	0.003	0.046
13:0	0.015	0.012	0.008	0.002	0.131
14:0	0.604 ^b	1.086 ^a	1.358 ^a	0.146	0.008
15:0	0.389	0.360	0.343	0.030	0.598
16:0	15.82 ^c	18.37 ^b	20.97 ^a	0.777	0.001
17:0	0.885	0.902	0.950	0.035	0.499
18:0	15.85	14.67	15.31	0.572	0.356
19:0	0.179	0.167	0.162	0.026	0.898
20:0	0.108	0.095	0.086	0.006	0.083
21:0	0.037 ^a	0.016 ^b	0.015 ^b	0.003	<0.001
22:0	0.251 ^a	0.211 ^{ab}	0.169 ^b	0.020	0.039
24:0	0.127	0.113	0.090	0.013	0.186
iso14:0	0.025	0.033	0.029	0.004	0.411
iso15:0	0.114	0.096	0.097	0.013	0.538
anteiso15:0	0.179	0.183	0.171	0.019	0.899
iso16:0	0.209	0.183	0.181	0.015	0.376
iso17:0	0.460	0.438	0.367	0.015	0.059
anteiso17:0	0.422	0.454	0.453	0.038	0.814
iso18:0 ^U	0.120	0.132	0.132	0.009	0.598
15:0DMA	0.100	0.077	0.052	0.007	0.053
AME1	0.095	0.119	0.094	0.015	0.359
AME2	0.105	0.083	0.064	0.013	0.105
16:0DMA	4.498	3.585	2.955	0.445	0.085
7c-16:1DMA	0.105 ^a	0.072 ^b	0.060 ^b	0.006	0.030
9c-16:1DMA	0.222 ^a	0.165 ^{ab}	0.111 ^b	0.012	0.009
17:0DMA	0.425	0.325	0.276	0.023	0.060
18:0DMA ^T	3.902 ^a	2.562 ^b	2.030 ^b	0.340	0.004
10t-18:1DMA	0.080	0.166	0.152	0.016	0.077
11t-18:1DMA	0.369 ^a	0.221 ^b	0.185 ^b	0.019	0.002
9c-18:1DMA	0.725 ^a	0.522 ^{ab}	0.380 ^b	0.042	0.015
11c-18:1DMA	0.078 ^a	0.062 ^{ab}	0.046 ^b	0.005	0.047
Pristanate	0.386 ^a	0.288 ^a	0.186 ^b	0.020	0.004
(2R,6R,10R,14)					
Phytanate1	0.049	0.051	0.053	0.004	0.885
(3R,7R,11R,15)					
Phytanate2	0.092 ^a	0.059 ^b	0.038 ^c	0.004	<0.001
(3S,7R,11R,15)					
17:0-cyclo ^V	0.065	0.075	0.055	0.007	0.099
∑SFA	36.19 ^b	37.71 ^{ab}	41.33 ^a	1.359	0.040
∑BCFA	1.526	1.514	1.432	0.101	0.811
∑AME	0.199	0.201	0.147	0.014	0.253
∑DMA	10.46 ^a	7.731 ^b	5.832 ^b	0.840	0.012
11t-18:1/10t-18:1DMA	5.37 ^a	1.43 ^b	1.78 ^b	0.29	<0.001

SFA = saturated fatty acid; BCFA = branched-chain fatty acid; AME = alk-1-enyl methyl ethers; DMA = dimethyl acetal.

Within a row, means without a common superscript differ ($P < 0.05$).

^TCoelution with 13c-16:1.

^UCoelution with 9t-18:1DMA.

^VCyclohexylundecanoic acid.

lipids were greater in grass-finished beef (10.5%; $P < 0.05$) and lowest in beef finished on concentrate for 2 months (5.8%), whereas the meat from animals finished on concentrate for 1 month had intermediate values (7.7%). The two major *trans*-18:1 DMA moieties in meat lipids were

Table 4 Cis-MUFA composition (percentages) of longissimus thoracis from bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing

Fatty acids (%)	Control	1 mo	2 mo	s.e.m.	P-value
9c-14:1	0.063 ^b	0.115 ^{ab}	0.171 ^a	0.026	0.036
7c-16:1 ^W	0.228 ^a	0.213 ^{ab}	0.194 ^b	0.009	0.046
9c-16:1	0.804 ^b	1.293 ^a	1.546 ^a	0.115	0.002
11c-16:1	0.056	0.055	0.064	0.007	0.646
12c-16:1	0.029	0.026	0.021	0.004	0.383
7c-17:1 ^X	0.138 ^a	0.107 ^b	0.068 ^c	0.010	0.001
9c-17:1	0.359 ^b	0.507 ^a	0.513 ^a	0.060	0.023
9c-18:1 ^Y	18.85	20.15	23.17	1.375	0.110
11c-18:1	1.435	1.340	1.281	0.059	0.232
12c-18:1	0.161 ^c	0.421 ^a	0.282 ^b	0.039	<0.001
13c-18:1	0.083 ^b	0.105 ^{ab}	0.143 ^a	0.014	0.022
14c-18:1	0.055	0.064	0.052	0.005	0.245
15c-18:1	0.058 ^b	0.110 ^a	0.101 ^a	0.010	0.005
9c-20:1	0.058	0.060	0.062	0.004	0.821
11c-20:1	0.059 ^b	0.088 ^a	0.090 ^a	0.008	0.033
13c-22:1	0.032	0.028	0.020	0.004	0.230
15c-24:1	0.028	0.050	0.040	0.006	0.081
∑cis-16:1	1.115 ^b	1.531 ^a	1.800 ^a	0.120	0.006
∑cis-18:1	20.64	22.18	25.03	1.352	0.103
∑cis-MUFA	22.40 ^b	24.59 ^{ab}	28.43 ^a	1.407	0.042
∑MUFA	27.88	31.98	34.27	1.697	0.056

MUFA = monounsaturated fatty acid; DMA = dimethyl acetal.

Within a row, means without a common superscript differ ($P < 0.05$).

^WCoelution with 9c-17:1DMA.

^XCoelution with 6d7d8c-18:1DMA.

^YCoelution with 10c-18:1.

10t-18:1 and 11t-18:1DMA, with 10t-18:1 increasing significantly in its relative percentage with increased length of feeding concentrate, whereas 11t-18:1 decreased significantly. The 11t-18:1/10t-18:1DMA ratio significantly decreased from approximately 5.4 to 1.5 with the feeding of concentrate (Table 3).

Significantly higher percentages of pristanic acid (2,6,10,14-tetramethylpentadecanoic acid) were found in meat from grass-fed bulls compared with concentrate-fed bulls and the same trend ($P < 0.001$) was observed for the phytanic acid isomer (3,5,7,11,15-tetramethylhexadecanoic acid), whereas no differences were detected for the 3,7,11,15 isomer (Table 3).

Cis-monounsaturated fatty acids

Total *cis*-monounsaturated fatty acids (MUFA) tended to be higher in meat from concentrate-finished than in grass-finished bulls (310 mg/100 g meat or 28% for 2 months concentrate finished; 212 mg/100 g of meat or 25% in 1 month concentrate finished; 126 mg/100 g of meat or 22% in grass finished; $P = 0.06$; Tables 2 and 4). On a percentage basis, 9c-16:1, 9c-18:1, and several other minor *cis*-MUFA isomers appeared to be higher in meat of animals fed concentrate.

Trans-monounsaturated fatty acids

Total *trans*-MUFA (mg/100 g meat) tended to be higher ($P = 0.053$; Table 2) in concentrate-fed bulls and this was

Table 5 Trans-MUFA composition (percentages) of longissimus thoracis from bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing

Fatty acids (%)	Control	1 mo	2 mo	s.e.m.	P-value
6 <i>t</i> /7 <i>t</i> -16:1	0.032	0.024	0.023	0.009	0.131
8 <i>t</i> -16:1	0.021 ^b	0.112 ^a	0.057 ^b	0.008	0.001
9 <i>t</i> -16:1	0.429 ^b	0.305 ^a	0.187 ^a	0.030	0.004
10 <i>t</i> -16:1	0.017	0.017	0.015	0.002	0.553
11 <i>t</i> /12 <i>t</i> -16:1	0.035 ^{ab}	0.037 ^a	0.026 ^b	0.003	0.038
4 <i>t</i> -18:1	0.017	0.020	0.014	0.003	0.518
5 <i>t</i> -18:1	0.014 ^b	0.024 ^a	0.016 ^{ab}	0.003	0.036
6 <i>t</i> /7 <i>t</i> /8 <i>t</i> -18:1	0.075 ^b	0.146 ^a	0.172 ^a	0.017	0.002
9 <i>t</i> -18:1	0.148 ^c	0.208 ^b	0.275 ^a	0.015	<0.001
10 <i>t</i> -18:1	0.291 ^b	2.824 ^a	2.280 ^a	0.422	0.001
11 <i>t</i> -18:1	2.410	1.756	1.841	0.335	0.364
12 <i>t</i> -18:1	0.125	0.171	0.155	0.019	0.250
13 <i>t</i> /14 <i>t</i> -18:1 ^z	0.360	0.436	0.352	0.038	0.214
15 <i>t</i> -18:1	0.098	0.118	0.117	0.014	0.524
16 <i>t</i> -18:1	0.133	0.123	0.094	0.017	0.276
11 <i>t</i> -20:1	0.051	0.045	0.026	0.008	0.095
13 <i>t</i> -20:1	0.010	0.017	0.013	0.003	0.293
∑ <i>trans</i> -16:1	0.533 ^a	0.496 ^a	0.308 ^b	0.031	0.007
∑ <i>trans</i> -18:1	3.671 ^b	5.826 ^a	5.316 ^a	0.509	0.020
∑ <i>trans</i> -MUFA	4.249 ^b	6.363 ^a	5.747 ^{ab}	0.509	0.024
∑ <i>trans</i> -FA	5.001 ^b	7.061 ^a	6.371 ^a	0.509	0.013
11 <i>t</i> -18:1/10 <i>t</i> -18:1	8.134 ^a	0.780 ^b	1.299 ^b	0.491	<0.001

MUFA = monounsaturated fatty acids; CLA = conjugated linoleic acid.

Within a row, means without a common superscript differ ($P < 0.05$).

trans-FA: *trans*-MUFA + *c,c*-dienes/trienes and *c,t*-dienes/trienes, but not CLA.

^zCoelution with 6-8*c*-18:1.

due to higher levels of several individual *trans*-18:1 isomers of which 10*t*-18:1 predominated. The second most abundant *trans*-18:1 isomer was VA, but it was not found to be different across treatments. On a percentage basis, total *trans*-MUFA and total *trans*-18:1 levels were significantly higher in meat from concentrate compared with grass-finished bulls (Table 5), although there was no apparent trend related to the length of concentrate feeding. The percentage of total *trans*-16:1 decreased with concentrate finishing.

With regard to individual *trans*-18:1 isomers, 10*t*-18:1 and 11*t*-18:1 together represented 76% of total *trans*-18:1 content. The longissimus muscle from bulls finished on grass had a significantly higher 11*t*-18:1/10*t*-18:1 ratio (8.13, $P < 0.001$) compared with concentrate-finished bulls (1.04). When the amounts of the individual *trans*-18:1 isomers were presented as a relative percentage of the total *trans*-18:1 content (Figure 1a), significantly higher relative proportions of 11*t*-18:1, 13*t*/14*t*-18:1 and 16*t*-18:1 were observed in grass-finished compared with concentrate-finished beef. In contrast, higher relative proportions of 10*t*-18:1 were consistently observed in concentrate-finished beef and this was accompanied with higher levels of 6*t*/7*t*/8*t*-18:1 and 9*t*-18:1.

PUFAs and other dienes

Linoleic acid was the major n-6 PUFA, and it was higher in concentrate-finished beef, especially in meat from animals

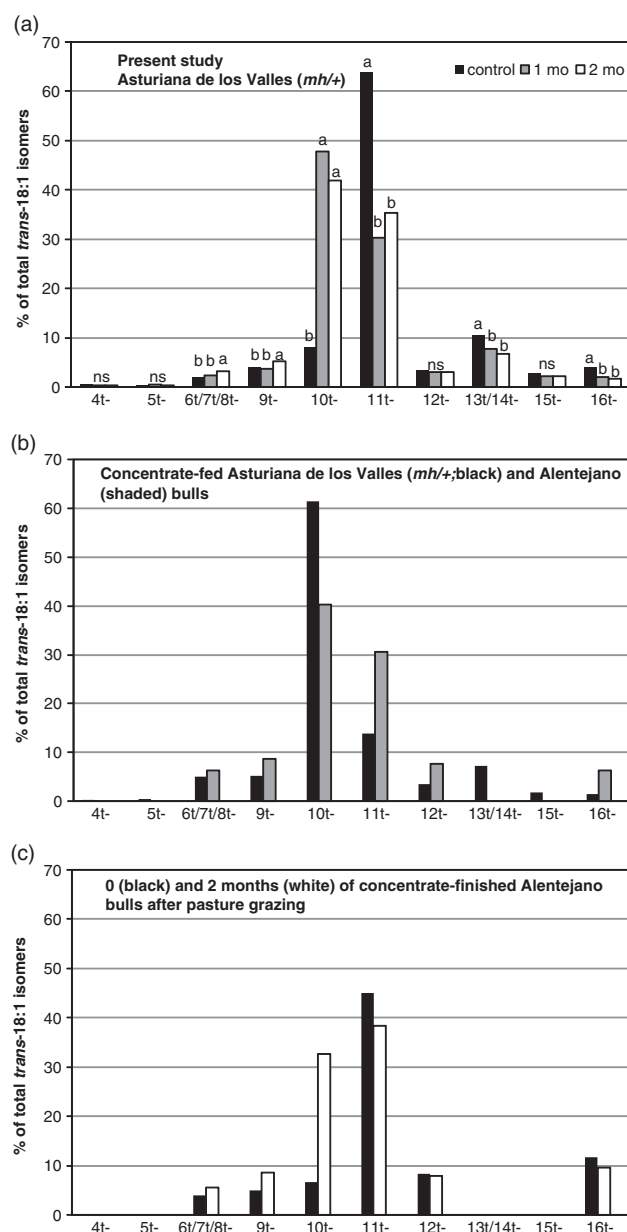


Figure 1 Relative isomeric distribution of individual *trans*-18:1 isomers of longissimus muscle from (a) the present study; 0 (control), 1 and 2 months of concentrate finishing after grazing, (b) concentrate-fed 'Asturiana de los Valles' (Aldai *et al.*, 2010a) and Alentejano purebred bulls (Alfaia *et al.*, 2009) and (c) 0 and 2 months of concentrate-finished Alentejano purebred bulls after pasture grazing (Alfaia *et al.*, 2009). Columns without a common superscript differ ($P < 0.05$).

fed concentrate for 2 months (103 mg/100 g of meat, $P < 0.05$; Table 2) compared with grass-finished beef (76.5 mg/100 g of meat). However, in general, the increased 18:2n-6 content did not result in the accumulation of elongation and desaturation products of 18:2n-6 (n-6 highly unsaturated fatty acid (HUFA)). When the data were expressed on a percentage basis, no significant differences were observed between these feeding strategies (Table 6).

Meat from bulls finished on grass or on 1 month of concentrate finishing showed the highest absolute contents of

Table 6 PUFA composition (percentages) of longissimus thoracis from bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing

Fatty acids (%)	Control	1 mo	2 mo	s.e.m.	P-value
Methylene interrupted					
18:2n-6	14.55	13.05	11.30	1.261	0.239
18:3n-6	0.058	0.062	0.049	0.007	0.391
20:3n-6	0.742	0.656	0.546	0.081	0.283
20:4n-6	3.249	3.199	2.346	0.410	0.242
22:2n-6	0.025	0.020	0.016	0.004	0.246
22:4n-6	0.178	0.172	0.124	0.022	0.199
22:5n-6	0.039 ^a	0.023 ^b	0.014 ^b	0.005	0.013
18:3n-3	3.470 ^a	2.214 ^b	1.340 ^c	0.233	<0.001
20:3n-3	0.045 ^a	0.023 ^b	0.017 ^b	0.002	<0.001
20:5n-3	1.016	0.924	0.731	0.139	0.377
22:5n-3	1.304	1.146	0.887	0.163	0.235
22:6n-3	0.094	0.073	0.075	0.015	0.596
∑PUFA	24.77	21.56	17.45	2.174	0.095
∑n-6 PUFA	18.85	17.18	14.40	1.720	0.227
∑n-3 PUFA	5.929 ^a	4.381 ^b	3.051 ^b	0.524	0.005
∑n-6 HUFA	4.189	4.032	2.833	0.303	0.178
∑n-3 HUFA	2.448	2.157	1.630	0.188	0.252
P/S	0.704	0.604	0.414	0.048	0.081
n-6/n-3 PUFA	3.292 ^b	4.010 ^{ab}	4.872 ^a	0.331	0.014
n-6/n-3 HUFA	1.862	1.906	1.822	0.069	0.879
Other dienes					
11 <i>t</i> ,15 <i>t</i> -18:2	0.028 ^b	0.058 ^a	0.045 ^{ab}	0.007	0.021
9 <i>t</i> ,12 <i>t</i> -18:2	0.043	0.053	0.038	0.006	0.134
9 <i>c</i> ,13 <i>t</i> -18:2	0.131	0.147	0.129	0.013	0.528
8 <i>t</i> ,13 <i>c</i> -18:2	0.084	0.082	0.074	0.007	0.563
9 <i>c</i> ,12 <i>t</i> -18:2	0.085	0.083	0.074	0.005	0.263
<i>c</i> , <i>t</i> -18:2unk1	0.054 ^b	0.086 ^a	0.063 ^b	0.007	0.007
9 <i>t</i> ,12 <i>c</i> -18:2	0.066	0.071	0.067	0.005	0.687
11 <i>t</i> ,15 <i>c</i> -18:2	0.263	0.308	0.286	0.046	0.789
<i>c</i> , <i>c</i> -18:2unk2	0.081 ^a	0.045 ^b	0.043 ^b	0.005	<0.001
9 <i>c</i> ,15 <i>c</i> -18:2	0.067	0.060	0.078	0.007	0.180
9 <i>c</i> ,11 <i>t</i> ,15 <i>c</i> -18:3	0.149	0.138	0.098	0.018	0.144
∑ <i>t</i> , <i>t</i> , <i>c</i> , <i>t</i> & <i>c</i> , <i>c</i> -dienes	0.738	0.803	0.745	0.052	0.604

PUFA = polyunsaturated fatty acid; HUFA = highly unsaturated fatty acids; P/s = polysaturated/saturated; unk = unknown position of double bonds.

Within a row, means without a common superscript differ ($P < 0.05$).

n-6 HUFA: sum of 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-6.

n-3 HUFA: sum of 20:3n-3, 20:5n-3, 22:5n-3 and 22:6n-3.

linolenic acid (17.3 mg/100 g of meat, $P < 0.05$) compared with bulls finished on concentrate for 2 months (12.5 mg/100 g of meat; Table 2). But again, the increased dietary content of 18:3n-3 did not result in increased levels of n-3 HUFA metabolites. On a percentage basis, 18:3n-3 ($P < 0.001$) and total n-3 PUFA ($P < 0.01$) were significantly higher in grass-finished beef, but did not result in increased levels of n-3 HUFA metabolites (Table 6). As expected, concentrate finishing resulted in a higher n-6/n-3 ratio ($P < 0.05$) compared with grass-finished beef, but this difference was mainly due to the diet differences in the precursor essential fatty acids, 18:2n-6 and 18:3n-3. When these were excluded the n-6 to n-3 HUFA ratio showed no differences among the feeding strategies.

Table 7 CLA composition (percentages) of longissimus thoracis from bulls fed concentrate-finishing diets for 0 (control), 1 or 2 months (mo) after pasture grazing

Fatty acids (%)	Control	1 mo	2 mo	s.e.m.	P-value
9 <i>c</i> ,11 <i>t</i> -18:2	0.306	0.249	0.322	0.050	0.532
7 <i>t</i> ,9 <i>c</i> -18:2	0.018 ^c	0.029 ^b	0.038 ^a	0.003	<0.001
8 <i>t</i> ,10 <i>c</i> -18:2	0.013	0.012	0.013	0.002	0.849
9 <i>t</i> ,11 <i>c</i> -18:2	0.035	0.026	0.030	0.004	0.308
10 <i>t</i> ,12 <i>c</i> -18:2	0.002 ^b	0.028 ^a	0.019 ^a	0.005	0.009
11 <i>t</i> ,13 <i>c</i> -18:2	0.037 ^a	0.016 ^b	0.022 ^{ab}	0.005	0.041
12 <i>t</i> ,14 <i>c</i> -18:2	0.002	0.002	0.002	0.000	0.853
12 <i>t</i> ,14 <i>t</i> -18:2	0.008	0.007	0.006	0.001	0.492
11 <i>t</i> ,13 <i>t</i> -18:2	0.026	0.023	0.017	0.004	0.279
10 <i>t</i> ,12 <i>t</i> -18:2	0.008	0.007	0.005	0.002	0.708
9 <i>t</i> ,11 <i>t</i> -18:2	0.009	0.010	0.010	0.001	0.657
8 <i>t</i> ,10 <i>t</i> -18:2	0.004	0.004	0.003	0.001	0.224
7 <i>t</i> ,9 <i>t</i> -18:2	0.002	0.002	0.002	0.000	0.754
12 <i>c</i> ,14 <i>t</i> -18:2	0.001	0.001	0.001	0.000	0.660
11 <i>c</i> ,13 <i>t</i> -18:2	0.007	0.006	0.006	0.001	0.791
9 <i>c</i> ,11 <i>c</i> -18:2	0.001 ^b	0.004 ^a	0.004 ^{ab}	0.001	0.045
∑ <i>t</i> , <i>t</i> -CLA	0.056	0.053	0.044	0.006	0.375
∑ <i>c</i> , <i>t</i> -CLA	0.421	0.369	0.454	0.055	0.530
∑CLA	0.478	0.426	0.501	0.060	0.642

CLA = conjugated linoleic acid.

Within a row, means without a common superscript differ ($P < 0.05$).

A number of *t*/*t*-18:2, *c*/*t*-18:2 and *c*/*c*-18:2 isomers derived either from 18:3n-3 (11*t*,15*t*-18:2, 11*t*,15*c*-18:2, 9*c*,13*t*-18:2 and 9*c*,15*c*-18:2) or from 18:2n-6 (9*t*,12*t*-18:2, 9*c*,12*t*-18:2 and 9*t*,12*c*-18:2), which elute between 19:0 and 20:0 during GLC analysis, were observed but very few differences were observed on a percentage basis (Table 6).

CLAs

RA (9*c*,11*t*-18:2) was the major CLA isomer with an average content of 2.5 mg/100 g of meat across treatments (Table 2) and represented 60% of the total CLA. Most of the remaining 40% was made up of 7*t*,9*c*-18:2, 9*t*,11*c*-18:2 and 11*t*,13*c*-18:2 (Table 7).

Concentrate-finished beef had significantly higher percentages of 7*t*,9*c*-18:2 ($P < 0.001$), 10*t*,12*c*-18:2 ($P < 0.01$) and 9*c*,11*c*-18:2 ($P < 0.05$), whereas grass-finished beef had higher percentages of 11*t*,13*c*-18:2 ($P < 0.05$). When individual CLA isomers were presented on a relative percent basis, the total CLA content of 7*t*,9*c*-18:2, 10*t*,12*c*-18:2 and 9*c*,11*c*-18:2 represented significantly higher proportions in concentrate-finished beef, whereas 11*t*,13*c*-18:2, 12*t*,14*t*-18:2 and 11*t*,13*t*-18:2 represented significantly higher proportions in grass-finished beef (data not shown).

Discussion

The type of animals used in the present study ('Asturiana de los Valles') were very lean as previously reported (Aldai *et al.*, 2007a; Martínez *et al.*, 2010). This cattle breed has a mutation in the bovine myostatin gene that is responsible for

the double-muscling phenotype (Grobet *et al.*, 1998), and animals used in this study were all heterozygous for the presence of the *mh* allele (*mhl* +). The double-muscling syndrome is an inherited condition, and is found in many breeds of cattle. It is also associated with many physical, physiological and histological characteristics, and meat obtained from these animals is reported to be leaner (see review by Arthur, 1995).

The intramuscular fat content was shown to be directly related to the extent of the concentrate-finishing period as reviewed by Wood *et al.* (2008). The accretion of fat in beef animals was reported to be mainly due to increased triacylglycerol, not phospholipids, which would result in higher percentages of SFA and MUFA relative to PUFA in the finished beef (Rule *et al.*, 1995). The higher percentages of long-chain SFA observed in grass-finished beef (Table 3) are consistent with previous reports of ruminants (Dugan *et al.*, 2007) suggesting that long-chain SFA are associated with higher intake of grass/pasture that is known to contain waxy cuticle rich in long-chain esters compared with grain-based concentrates (Post-Beittenmiller, 1996). Concentrate diets provide, in general, increased availability of oleic and linoleic acids that are major fatty acids in cereal grains (Table 1), which results in more linoleic acid on an absolute basis in the meat from concentrate-finished bulls. In addition, higher contents of metabolites derived from 18:2n-6, such as the methylene-interrupted 18:2 (9*t*,12*t*-18:2, 9*t*,12*c*-18:2), CLA (10*t*,12*c*-18:2) and 18:1 (10*t*-18:1) isomers, were also observed. On the other hand, as noted by Enser *et al.* (1998), pastures are a good source of linolenic acid (18:3n-3; Table 1), which explains the inverse relationship between concentrate-finishing time and muscle levels of 18:3n-3. Recognized metabolites of 18:3n-3, including trienes (9*c*,11*t*,15*c*-18:3), dienes (11*t*,15*c*-18:2, 9*c*,13*t*-18:2 and 9*c*,15*c*-18:2), CLA (11*t*,13*c*-18:2, 11*t*,13*t*-18:2 and 12*t*,14*t*-18:2) and monoenes (13*t* to 16*t*-18:1), were not different across finishing strategies. These rumen metabolites of PUFA were previously identified in a number of studies (Kraft *et al.*, 2003; Cruz-Hernandez *et al.*, 2004 and 2006; Destailats *et al.*, 2005; Bessa *et al.*, 2007; Gomez-Cortes *et al.*, 2009).

Trans-MUFA, and particularly the *trans*-18:1 isomers, are the major intermediates which accumulate during biohydrogenation of PUFA (i.e. 18:2n-6, 18:3n-3; Bessa *et al.*, 2000). The significantly higher absolute and relative contents of *trans*-18:1 found in concentrate-finished beef compared with grass-finished beef were mainly due to higher contents of 6*t*/7*t*/8*t*-18:1, 9*t*-18:1 and 10*t*-18:1 as observed by Alfaia *et al.* (2009) and Leheska *et al.* (2008). High contents of 10*t*-18:1 have been observed in tissues of concentrate-fed ruminants (Aldai *et al.*, 2008b and 2010b), whereas 11*t*-18:1 has been consistently associated with forage feeding in beef (Bessa *et al.*, 2006; Dugan *et al.*, 2008; Kraft *et al.*, 2008; Alfaia *et al.*, 2009; Figure 1). In Figure 1b, the *trans*-18:1 profile of concentrate-fed bulls from two studies (Alfaia *et al.*, 2009; Aldai *et al.*, 2010a) using different breeds are presented for comparison purposes. In both studies, 10*t*-18:1 was clearly the major isomer. In Figure 1c, the *trans*-18:1 profile of beef from bulls

concentrate-finished for 0 and 2 months after pasture grazing is presented (Alfaia *et al.*, 2009).

As observed in Table 5, with the exception of 9*t*-18:1, there were no significant differences in *trans* isomers in the meat lipids when comparing concentrate-finished animals (i.e. 1 month compared with 2 months). These results show that certain changes in meat lipid composition were evidently complete after 1 month of feeding. The reason for the increased content of 10*t*-18:1 could be related to a decrease in rumen pH and associated bacteria changes (Harfoot and Hazlewood, 1997), whereas ruminal vitamin E content associated with the grass intake could have also been a potential reason for the improvement of the 11*t*-18:1/10*t*-18:1 ratio (Pottier *et al.*, 2006; Juárez *et al.*, 2010).

The n-3 content in the meat of grass-fed animals in this study was high in comparison with others (Ponnampalam *et al.*, 2006; Kraft *et al.*, 2008), whereas the n-6/n-3 was also higher (3.3) than reported by Enser *et al.* (1998; 2.0 to 2.3 for British cattle), Nuernberg *et al.* (2002; 1.3 for German Simmental bulls and Holstein steers) and Alfaia *et al.* (2009; 1.8 for Alentejano purebred). These differences could be in part explained by differences in breed (Raes *et al.*, 2003; Aldai *et al.*, 2007b; Kraft *et al.*, 2008), forage species (Collomb *et al.*, 2002; Fraser *et al.*, 2009) and/or stage of pasture maturity (Dewhurst *et al.*, 2001; Vanhatalo *et al.*, 2007), or the relatively high content of 18:2n-6 in the concentrate. The lack of increased desaturation and elongation metabolites from 18:2n-6 (substrate) and 18:3n-3 (competition) in this breed of cattle was surprising. However, it has been recently demonstrated that the expression of some of the genes involved in lipid metabolism is inhibited in the *mhl/mh* genotype of 'Asturiana de los Valles' (Perez *et al.*, 2010), which might also apply to the *mhl* + genotype. The lack of response could also be due to the limited length of feeding (60 days in this study). Duckett *et al.* (1993) started to observe changes in meat lipids of Angus × Hereford steers after 56 days of concentrate feeding. Furthermore, an alternate explanation could reflect low enzyme activity (Δ^9 -desaturase and elongase enzymes) in this particular genotype supported by previous observations of Aldai *et al.* (2008a). Similar results of decreased desaturation and elongation of the essential fatty acids were observed in the Limousine breed fed concentrate diets for a much longer period (Kraft *et al.*, 2008). The overall low CLA content is also evidence of low Δ^9 -desaturase activity within the muscle. Across treatments, there were slight differences in some of the individual CLA isomers, similar to those observed by Dannenberger *et al.* (2005) in beef and Dugan *et al.* (2007) in muskox, where 11*t*,13*c*-18:2 was the second most abundant isomer in grass-finished, and 9*t*,11*c*-18:2 and 10*t*,12*c*-18:2 in concentrate-fed animals. The 11*t*,13*c*-18:2 isomer is a metabolite of 18:3n-3, which has been linked to the isomerization of 11*t*,15*c*-18:2 (Fukuda *et al.*, 2009).

The plasmalogens in the beef intramuscular fat are seldom discussed, mainly because total beef lipids are methylated either using base catalysts to avoid isomerization of CLA (Kramer *et al.*, 1997) that does not break the alk-1-enyl bond

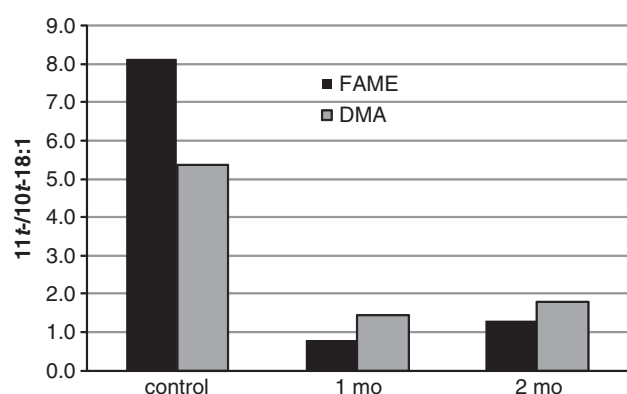


Figure 2 Fatty acid methyl ester and dimethylacetal 11t-18:1/10t-18:1 ratio of *longissimus thoracis* from bulls with 0 (control), 1 or 2 months (mo) of concentrate finishing after pasture grazing.

or using acid catalysts, but the DMAs formed are generally not reported (Cruz-Hernandez *et al.*, 2006). The current results show an increased content of plasmalogens in pasture-fed compared with concentrate-fed beef, which is in agreement with two other studies showing that pasture feeding results in increased amounts of plasmalogens in muscle lipids of beef (Dannenberger *et al.*, 2005; Kraft *et al.*, 2008). The fact that the plasmalogenic content can also be affected by breed (Kraft *et al.*, 2008) makes it difficult to compare results between studies and the question of which factors affect the plasmalogenic content in muscle is difficult to assess. Concentrate finishing, which increases the dietary fat content, did not affect the absolute amount of plasmalogenic lipids in muscle, but did significantly reduce the relative abundance ($P < 0.05$). This is understandable as increased levels of fat in muscle tissue are mainly associated with triacylglycerols and not phospholipids, and plasmalogens being phospholipids would thus not be affected. We also considered whether different dietary fatty acids, such as 18:2n-6 and 18:3n-3, could affect the plasmalogenic content in muscle tissue, even though these unsaturated fatty acids are not incorporated into plasmalogenic lipids (Horrocks, 1972; Wolff, 2002). It would appear, however, that increased dietary 18:3n-3 is generally associated with higher levels of plasmalogens, but this should be confirmed by comparing appropriate diets using the same breed. The results of this study clearly showed that the *trans* fatty acid metabolites formed by rumen bacteria were incorporated into plasmalogenic lipids (Table 3), as was previously demonstrated by Wolff (2002). The ratio of the 11t-18:1/10t-18:1 FAME and DMA moieties are shown in Tables 2 and 3, respectively, and are presented for comparison in Figure 2. VA (11t-18:1) predominated over 10t-18:1 in muscle tissue from pasture-fed beef when expressed as mg/100 g or as relative %; the ratio was higher for FAMEs (about 8) than for DMAs (about 5). On the other hand, the 11t-18:1/10t-18:1 ratio was similar (about 1) for both FAMEs and DMAs whether expressed as mg/100 g or relative %, and were not significantly different between 1 and 2 months of feeding the concentrate.

Phytanic acid is a multibranched-chain fatty acid (MBCFA) originating from the phytol side-chain of chlorophyll, which is hydrolyzed and oxidized by ruminal bacteria (Patton and Benson, 1966). It is a normal constituent of the tissues, milk and plasma of ruminants (Lough, 1977). In cattle, the proportion of phytanic acid has been found to vary widely depending on the composition of the feed ingested (Lough, 1977). Pristanic acid is also an MBCFA derived from the α -oxidation of phytanic acid and it also accumulates in tissues (Ferdinandusse *et al.*, 2002). Even though MBCFAs are related to higher grass intake (i.e. leafy chlorophyll-rich material), and has been associated with prostate cancer risk (Xu *et al.*, 2005), we did not find any differences in MBCFA on an absolute basis.

Overall, the beef intramuscular fat content was very low and it could in fact be classified as 'lean meat' (<5%). If we compare the profile against nutritional recommendations, all finishing strategies were close to or above 0.45 for the polysaturated/saturated (P/S) ratio, whereas meat only from grass-finished or concentrate-finished bulls for 1 month achieved the recommended n-6/n-3 ratios of less than 4. In several countries such as Denmark, Canada and the United States, recommendations for human health have now come to include reduced *trans* fat (*trans*-FA) intake, as their consumption has been linked to health issues (Mensink *et al.*, 2003; Odegaard and Pereira, 2006). On the other hand, ruminant fats are exempt from *trans* labelling requirements because these sources are considered to be 'natural' and therefore assumed to be 'healthy' (mainly VA and RA; Ratnayake and Zehaluk, 2005). For regulatory purposes, *trans*-FAs are defined as *trans* monoenes plus other fatty acids containing isolated *trans* double bonds, except *trans*-containing CLA. Any food containing less than 0.2 g *trans*-FA per serving (or 2% of total fat content) in Canada, and less than 0.5 g *trans*-FA per serving in the USA are considered free of *trans*-FA. The beef obtained in this study had a maximum content of 0.14 g *trans*-FA/100 g of meat that was well below the limit set either in Canada or in the USA.

Conclusions

The beef obtained from the studied finishing strategies was within or close to the recommended values for human consumption (i.e. P/S > 0.45, n-6/n-3 < 4.0), and the total *trans*-FA content was low. However, the results indicated that finishing had a major impact on *trans*-18:1 and CLA isomeric profile, with grass finishing judged to provide a healthier beef fatty acid profile, whereas 2 months of concentrate finishing negatively affected the *trans*-18:1 and CLA isomer composition. Overall, these results reinforce evidence that beef from pasture-fed animals had the highest nutritional quality, whereas 2 months of concentrate feeding produced a significant reduction in the desirable fatty acids such as VA and RA and gave rise to many undesirable *trans*-18:1, 18:2 and CLA isomers. However, when working with lean breeds an increased forage-to-concentrate ratio will likely be necessary to maintain high levels of desirable

and low levels of undesirable fatty acids while achieving good production efficiencies (e.g. carcass fatness) and consumer acceptance (e.g. juiciness) of the final meat. It remains a challenge to sufficiently increase intramuscular fat in this extremely lean genotype to achieve consumer acceptance and to meet beef grade standards, and at the same time maintain or preferably increase the content of healthful fatty acids. The lack of response of increased levels of 18:2n-6 and 18:3n-3 to produce their long-chain PUFA metabolites in this breed is unique and indicates that a strategy to increase their content cannot be achieved through dietary increase of these essential fatty acids.

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